Preservation of Native Collagen

J. PIKKARAINEN and E. KULONEN

Department of Medical Chemistry, University of Turku, Turku 3, Finland

When collagenous material was kept immersed in an equal volume of 30 % (v/v) ethanol at +5°C, the amount of collagen soluble in salt solution decreased appreciably but the thermal shrinking temperature and the starch-gel electrophoretic pattern of the components remained normal.

When samples of the skins of various animals were collected for the comparison of collagens, decomposition during transport was avoided by the immersion the tissues in aqueous ethanol. We assessed the effects of the ethanol concentration and the temperature with samples from an adult flounder (*Pleuronectus flesus*).

EXPERIMENTAL

The fishes were obtained alive. The skin was stripped off or the whole fish cut into pieces. One sample of the material was studied immediately, but others samples were immersed in an equal volume of 30 % or 60 % (v/v) aqueous ethanol and stored for 3—4 days either at room temperature (about +25°C) or at +5°C.

The solubility of collagen in salt and acid buffer solutions was studied by carrying out three consecutive extractions of the homogenized material with a ten-fold volume of 0.45 *M* sodium chloride[1] overnight in the cold room followed by four extractions with a ten-fold volume of 0.15 *M* citrate buffer of pH 3.7[2]. The hydroxyproline contents of the extracts were determined according to Woessner.[3]

RESULTS

The storage in ethanol decreased the NaCl-soluble fraction of skin collagen from 6.4 % to 0.7—2.7 % depending on the conditions. The storage did not cause any significant change in the proportion of collagen soluble in citrate buffer from the average 24%. The concentration of ethanol or the temperature were not significant.

The fraction soluble in citrate buffer was purified by precipitation with sodium chloride (final concn. 15 %, w/v), repeated extraction of the precipitate with citrate buffer, centrifugation at 160,000 *g* for 30 min, and dialysis of the supernatant against 0.02 *M* disodium phosphate. Samples of this purified collagen were denatured for 15 min at +40°C and the components studied by starch-gel electrophoresis.[4,5] The characteristic pattern was not obtained with the preparations from materials which had been stored in ethanol at room temperature but was obtained with preparations from materials kept at +5°C (Fig. 1).

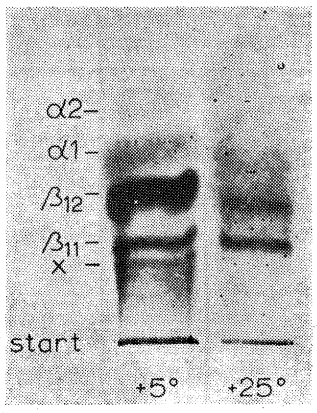

Fig. 1. Starch-gel electrophoretic pattern of denatured acid-soluble collagen from flounder skin which had been stored in ethanol either at +5°C or at room temperature (about +25°C). Identification of the α2-component is questionable.

The storage of the material in ethanol at +5°C had no effect on the thermal shrinking temperature which remained the same as for the fresh samples (+51—+53°C).

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